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PROTEIN RELATIONSHIPS AMONG TITMICE (*PARUS*)

FRANK B. GILL,[1] DAVID H. FUNK,[1] AND BENGT SILVERIN[2]

ABSTRACT.—As a first step toward understanding the evolutionary and biogeographical relationships among species of *Parus,* we compared allozymes at 34 loci of nine North American and six Eurasian species representing six subgenera. The results of this electrophoretic survey provide the first broad summary of genetic relationships among species of the genus *Parus.* Distance Wagner and UPGMA analyses suggest that: (1) the crested North American titmice (subgenus *Baeolophus*) are only distantly related to the other parids examined; (2) the Bridled Titmouse (*P. wollweberi*) is closest to *Baeolophus* titmice and convergent in appearance to the Crested Tit (*P. cristatus*); (3) the Marsh Tit (*P. palustris*) and Willow Tit (*P. montanus*) probably are sister taxa, but the Carolina Chickadee (*P. carolinensis*) and Black-capped Chickadee (*P. atricapillus*) may not be; (6) the Black-capped Chickadee is genetically closer to the Mountain Chickadee (*P. gambeli*) and Mexican Chickadee (*P. sclateri*) than to the Carolina Chickadee and (7) the Boreal Chickadee (*P. hudsonicus*) and Chestnut-backed Chickadee (*P. rufescens*) are sister taxa related in turn to the *atricapillus* species group.

The titmice of the world (Paridae) are a well-defined taxonomic group. All but two of the 46 species are classified in the genus *Parus* (Snow 1967). The 11 North American species apparently are descendents of Eurasian lineages that crossed the Bering land bridge during interglacial epochs of the Pleistocene (Mayr 1946, Parkes 1958). Following range expansions in North America, the populations of some of these colonists underwent repeated fragmentation and vicariant speciation due to the advance and retreat of the glaciers (Selander 1965, Brewer 1963, Dixon 1978). The current view of relationships among species is suggested by the subgeneric classification of Thielcke (1968) (Appendix I). The three large, crested, North American titmice (Plain Titmouse, *P. inornatus*; Tufted Titmouse, *P. bicolor*; and Black-crested Titmouse, *P.* [*bicolor*] *atricristatus*) constitute the subgenus *Baeolophus.* The Bridled Titmouse (*P. wollweberi*) is an enigmatic species assigned to the subgenus *Lophophanes* with two Old World species, the Crested Tit (*P. cristatus*) and the Gray-crested Tit (*P. dichrous*). The North American chickadees (subgenus *Poecile*) include a "brown-capped" superspecies (Chestnut-backed Chickadee, *P. rufescens*; Boreal Chickadee, *P. hudsonicus*; Siberian Tit, *P. cinctus*), and a "black-capped" species group (Black-capped Chickadee, *P. atricapillus*; Carolina Chickadee, *P. carolinensis*; Mountain Chickadee, *P. gambeli*; and Mexican Chickadee, *P. sclateri*) (Mayr and Short 1970). Superficially, these

[1] The Academy of Natural Sciences, Philadelphia, Pennsylvania 19103, and [2] Dept. Zoology, Univ. of Göteborg, Göteborg, Sweden.

"black-capped" chickadees appear close to certain Eurasian forms, especially the Willow Tit (*P. montanus*), with which the Black-capped Chickadee has been considered conspecific (see Snow 1956).

In this paper we explore the relationships among North American chickadees and titmice and selected Eurasian species based on electrophoretic surveys of genetic loci that code for enzymes that function in intermediary metabolism. Differences among species allow us to construct hypotheses of evolutionary relationship and biogeographical history (Wilson et al. 1977, Barrowclough 1983, Barrowclough et al. 1985, Nei 1987).

MATERIALS AND METHODS

We surveyed allozymes present in tissues of nine North American species and six Eurasian species of *Parus* (Appendix I). With the exception of Boreal Chickadee (N = 4) and the Coal Tit (*P. ater*) (N = 3), we used five individuals of each species, or in the case of the Black-capped Chickadee, five individuals from each of two geographically distant populations (*P. a. atricapillus* from Pennsylvania and *P. a. occidentalis* from Washington). A White-breasted Nuthatch (*Sitta carolinensis*) served as the outgroup, but in hindsight this nuthatch was too different genetically to help resolve most issues of character polarity.

Tissue samples were preserved on dry ice or liquid nitrogen in the field and transferred to freezer storage at −70°C until analysis. Methods were similar to those of Braun and Robbins (1986), except that we used horizontal rather than vertical starch gel electrophoresis. Most of the 34 presumptive genetic loci we examined (Appendix I) matched theirs. We could not obtain satisfactory results with four of their loci (Alat-1, Alat-2, Glud, Pro-1), which included diagnostic alleles, and we scored six additional loci (Ck-3, Pgm-2, Me, Np, Gda, Ald). Alleles at each presumptive genetic locus were scored with reference to their mobility from the origin, and labelled *a, b, c,* etc. in sequence from the one closest to the anode. Allelic frequencies were calculated from the individual genotypes scored from banding patterns on the gels.

We used the computer program BIOSYS-1 (Swofford and Selander 1981) for calculations of gene frequencies and genetic distances, as well as construction of distance Wagner trees and UPGMA phenograms based on both Rogers' (1972) and Cavalli-Sforza and Edwards' (C-S&E) (1964) chord distances. The analysis of evolutionary relationships based on electrophoretic surveys of allozyme compositions is controversial (Felsenstein 1982, 1983, 1984; Lanyon 1985; Farris 1986; Swofford and Berlocher 1987). Distance Wagner trees and UPGMA clustering methods based on Rogers' (1972) genetic distances are the most commonly used procedures. Tests of the different tree building procedures by Nei et al. (1983) suggest that C-S&E chord distances produce the most accurate branching topologies in both distance Wagner trees and UPGMA phenograms. UPGMA phenograms give the most accurate trees when small numbers of loci are analyzed, but assume constant evolutionary rates (Rohlf and Wooten 1988). We also used Felsenstein's (1981) unrooted maximum likelihood networks, specifically the CONTML program in PHYLIP 2.8. In modelling tests that assumed constant evolutionary rates, this approach produced the most accurate trees when large numbers of loci (e.g., over 50) are analyzed (Rohlf and Wooten 1988). Kim and Burgman (1988) also found maximum likelihood to perform better than either maximum parsimony or phenetic clustering in simulations with unequal evolutionary rates that corresponded to a genetic drift model with population bottlenecks, as may be appropriate for birds (Barrowclough et al. 1985). Character state polarities of alleles refer to their distributions among the hierarchy of clusters in the distance Wagner tree (Richardson et al. 1986).

RESULTS

Twelve of the 34 loci (35%) were monomorphic in *Parus,* including five that were monomorphic in both *Sitta* and *Parus* (*): Aat-2, Ck-1*, Ck-2, Pgm-1, Ldh-2, Mdh-1*, Mdh-2, Sordh*, Sod-2, Hb*, Mb, G3pdh*. Distinct alleles characterized *Sitta* at 26 loci. In *Parus,* fixed interspecific differences characterized 16 of the 22 informative loci. The number of alleles per locus averaged 1.1. Across species, an average of 9.2% of the loci were polymorphic (range = 0.0–17.6; 0.95 criterion).

Nei's (1978) genetic distances (*D*) between species pairs ranged from 0.005 to 0.396. Distances between subgenera, using Black-capped Chickadee to represent the subgenus *Poecile* and Tufted Titmouse to represent the subgenus *Baeolophus,* averaged three–four fold greater ($\bar{D} = 0.22 \pm 0.09$ SD, N = 15) than distances between species pairs of *Poecile* ($\bar{D} = 0.06 \pm 0.05$ SD, N = 28). Distances between the two species of *Baeolophus* titmice ($\bar{D} = 0.06$) were the same as the average among pairs of *Poecile* chickadees. Braun et al. (1984) reported the distance between Black-crested Titmouse and Tufted Titmouse to be $\bar{D} = 0.063$, the same as we found between Tufted Titmouse and Plain Titmouse. Distances between North American chickadees and *Baeolophus* titmice ($\bar{D} = 0.29 \pm 0.04$ SD, range 0.2–0.4) were higher than those estimated for all other combinations of species. The protein distance between Tufted Titmouse and Carolina Chickadee is 0.28, not 0.09 as erroneously reported for this pair of species by Mack et al. (1986). The Marsh Tit (*P. palustris*) was genetically the most divergent of all chickadees. Among North American species of chickadees, Carolina Chickadee and Mexican Chickadee were the most different. Chestnut-backed Chickadee and Boreal Chickadee were particularly close to one another ($\bar{D} = 0.004$) and to Black-capped Chickadee ($\bar{D} = 0.004, 0.007$). The two races of Black-capped Chickadee from opposite sides of the continent were virtually identical ($\bar{D} = 0.00$).

Relationships among subgenera.—Distance Wagner trees (Fig. 1), UPGMA phenograms, and the maximum likelihood network (Fig. 2) all suggested the following relationships among subgenera, regardless of whether Rogers' or C-S&E genetic distances were used: (1) the North American crested titmice ("*Baeolophus*" plus Bridled Titmouse) represent a distinct parid lineage; (2) Bridled Titmouse is not allied to the Crested Tit, rather it is convergent in appearance; (3) the Crested Tit is the closest of the Eurasian taxa to *Poecile* chickadees; (4) the Eurasian subgenera represent distinct lineages without clear affinities among themselves.

Two major ambiguities persist in the topological relationships among subgenera. First is the arrangement of Coal Tit (subgenus *Periparus*), Blue Tit (*P. caeruleus*) (subgenus *Cyanistes*), and Great Tit (subgenus *Parus*).

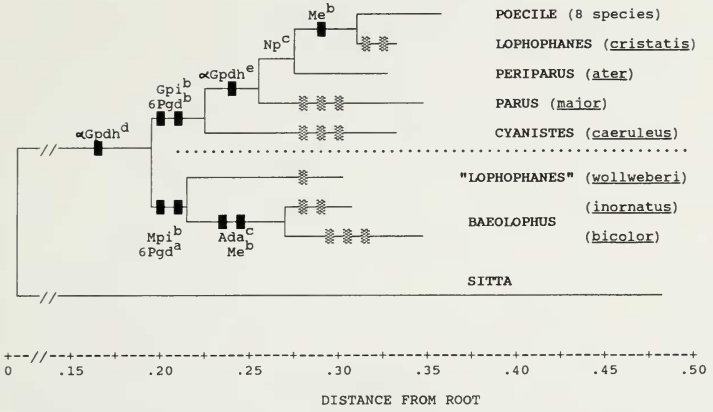

FIG. 1. Distance Wagner tree of relationships among subgenera of *Parus* using Rogers' genetic distances. See Fig. 3 and 4 for relationships among *Poecile* chickadees. The tree was rooted with *Sitta carolinensis*; branch lengths were not optimized. Black rectangles indicate shared, derived alleles (synapomorphies); Pepper rectangles indicate alleles unique to species (autapomorphies). Total length of tree = 1.68; percent standard deviation = 14.77.

Both distance Wagner trees based on C-S&E chord distances, and the shortest distance Wagner tree based on Rogers' distances, projected a hierarchical sequence of Blue Tit-Great Tit-Coal Tit, as illustrated in Fig. 1. A slightly longer, alternative distance Wagner tree based on Rogers' distance linked Coal Tit and Blue Tit, as did UPGMA phenograms based on both Rogers' and C-S&E distances. The second ambiguity concerns the relationship between the Crested Tit and *Poecile* chickadees. Distance Wagner trees and the maximum likelihood networks consistently placed the Crested Tit outside the cluster of *Poecile* species. UPGMA phenograms clustered the Crested Tit between Marsh Tit and Carolina Chickadee.

One to three unique alleles characterized all but one of the subgenera (Fig. 1). Fixed differences separated *Parus* from *Sitta* at 17 loci, and combinations of the Eurasian lineages from the North American crested titmice at five loci. At one of these five loci (Gpi), the North American crested titmice retained plesiomorphic allele *a* (also present in *Sitta*), whereas all other species had the alternative (derived) allele *b*. At the other four loci (Np, αGpdh, 6Pgd, Mpi), the alleles distinguishing the North American crested titmice differed from *Sitta* but still may have been plesiomorphic: (1) locus Np—the North American crested titmice shared allele *d* with Great Tit and Blue Tit, whereas other species exhibited the derived state *c*, or (Crested Tit only) the derived states *f* and *g*; (2) locus αGpdh—the North American crested titmice were united by allele

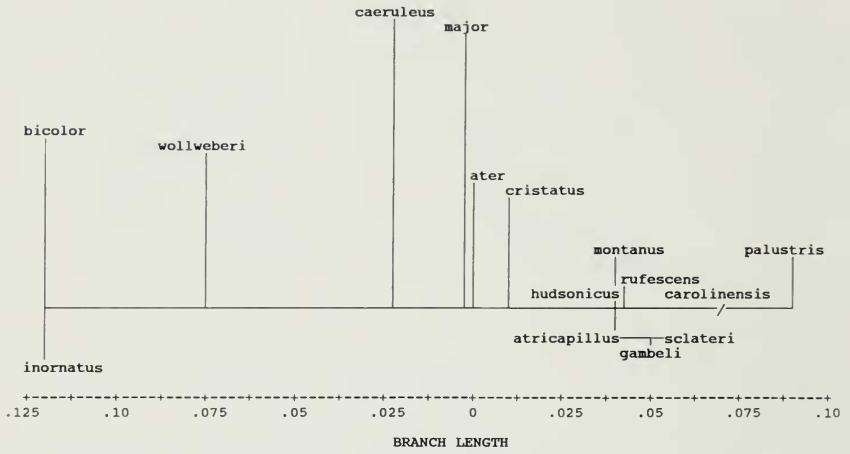

FIG. 2. Maximum likelihood network of relationships among species of *Parus*; 408 trees were examined. Ln likelihood = 1420.11.

d present also in Blue Tit, whereas all other species shared the derived allele *e*; (3) locus 6Pgd—the North American crested titmice were united by allele *a*, whereas all other species had allele *b* or (Great Tit and Blue Tit) allele *c*; and (4) locus Mpi—the North American crested titmice were united by allele *b*, which was present also (at low frequency) in Marsh Tit and Willow Tit. Possibly the distributions of $6Gpd^{b}$ and Mpi^{b} reflect symplesiomorphies, but better outgroup information is needed to resolve the interesting polarities at these loci.

Among the North American crested titmice, Tufted Titmouse and Plain Titmouse are sister taxa distinguished from Bridled Titmouse by derived alleles at two loci (Ada^{c}, Me^{b}). Braun et al.'s (1984) data suggest that the Black-crested Titmouse has the same allele as the Tufted Titmouse at one of these loci (Me). The other locus (Ada) was polymorphic in their samples of Black-crested Titmouse and Tufted Titmouse, with a strong frequency difference between the two species. The derived allele prevailed (96%) in Tufted Titmouse, whereas the primitive allele, which was fixed in Bridled Titmouse and all chickadees, prevailed in Black-crested Titmouse (92%). Allele *c* present as a polymorphism at a third locus (Pro-2) also distinguished Tufted Titmouse and Plain Titmouse from Bridled Titmouse, but the same (or an indistinguishable) allele was present in two species of chickadees (see below).

Few synapomorphies linked the other subgenera. Alleles at locus Me appear to link Bridled Titmouse and Crested Tit with the subgenus *Poecile* (Me^{b}) and Blue Tit with Great Tit (Me^{e}), but this variable locus requires further study. Allele *c* at locus 6Pgd also linked Great Tit and Blue Tit.

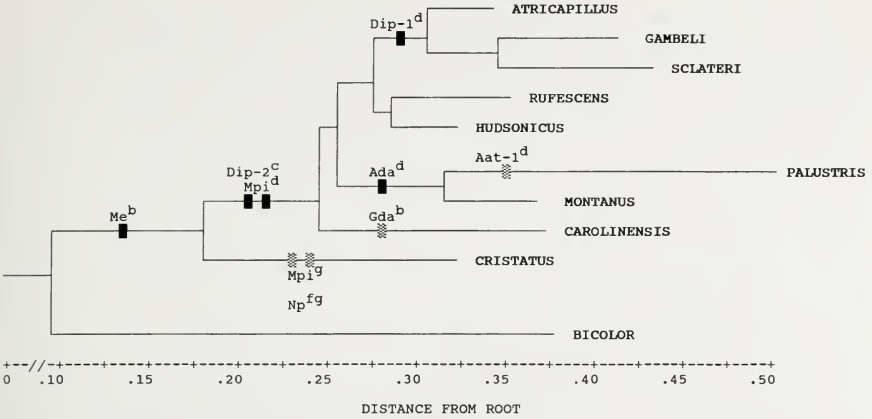

FIG. 3. Distance Wagner tree of genetic relationships among species of *Poecile* chickadees using Cavalli-Sforza and Edwards' genetic distances. This tree is a subset of the full set of species rooted with *Sitta,* but only *bicolor* is included here as an outgroup for reference (total length of full tree = 2.86; percent standard deviation = 10.99). Synapomorphies and autapomorphies indicated as in Fig. 1.

Relationships among chickadees.—Similar allozyme compositions characterized the *Poecile* chickadees, resulting in short branch lengths (Figs. 3 and 4) and topologies that varied with tree length and algorithms. Two species combinations, however, consistently clustered together: (1) Mexican/Mountain and (2) Boreal/Chestnut-backed. Marsh Tit and Willow Tit linked as sister taxa in distance Wagner trees, but not in the UPGMA phenograms or the maximum likelihood network, which positioned Marsh Tit outside all other *Poecile* species. Carolina Chickadee did not cluster with Black-capped Chickadee, but rather placed outside all other species in the distance Wagner trees, and outside all species, except Crested Tit and Marsh Tit, in the UPGMA phenograms and the maximum likelihood network. The distance Wagner trees and maximum likelihood network clustered Black-capped Chickadee most closely with Mountain Chickadee and Mexican Chickadee, but the UPGMA phenograms suggested a closer tie to the brown-capped species, Boreal Chickadee and Chestnut-backed Chickadee. Tentatively, we suggest that Fig. 3 represents the best available working hypothesis of relationships among chickadees.

Shared alleles distinguished some sets of species (Fig. 3). Me^b characterized Crested Tit (and Bridled Titmouse) plus all *Poecile* chickadees except Marsh Tit, which retained or reverted to allele *a.* $Dip\text{-}2^c$ distinguished the *Poecile* chickadees from all other subgenera. So did allele *d* at Me, with the caveat that Marsh Tit either retained or reverted to the

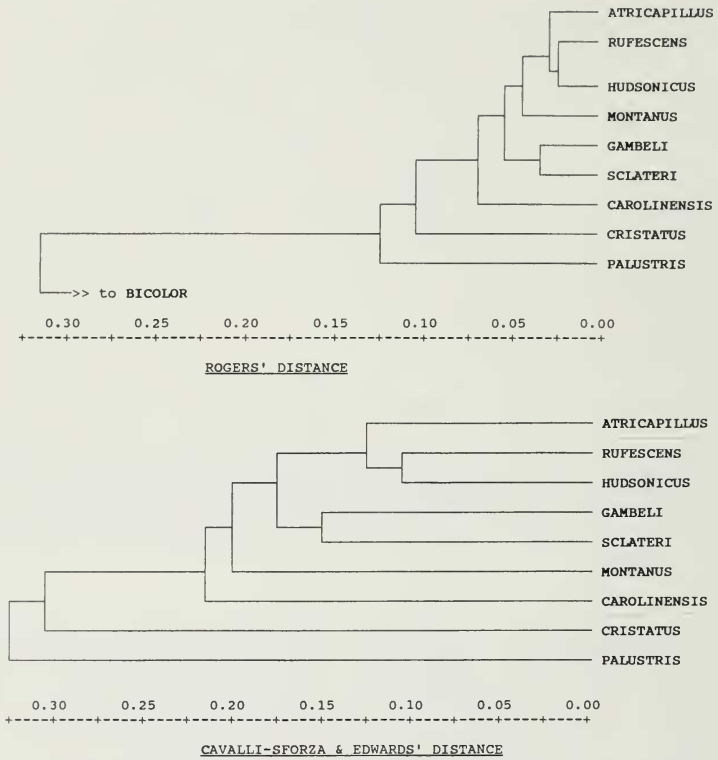

FIG. 4. UPGMA dendrograms of genetic relationships among species of *Poecile* chickadees using Rogers' genetic distances (above) and Cavalli-Sforza and Edwards' genetic distances (below). This diagram is a subset of the full set of all species of *Parus* examined. Percent standard deviation = 19.98; cophenetic correlation coefficient = 0.989.

primitive allele *b* which was present in the North American crested titmice. Black-capped Chickadee, Mountain Chickadee, and Mexican Chickadee shared a unique allele at the one locus (Dip-1^{d}). This allele was fixed in both Mountain Chickadee and Mexican Chickadee but not in Black-capped Chickadee, which retained the primitive allele (*c*). No unique states linked Boreal Chickadee and Chestnut-backed Chickadee. One synapomorphy (locus Ada^{d}) linked Willow Tit and Marsh Tit. Fixed autapomorphies at one locus each distinguished Carolina Chickadee (Gda^{b}) and Marsh Tit (Aat1^{d}) from all other chickadees.

DISCUSSION

The results of this electrophoretic survey provide the first broad summary of genetic relationships among species of the genus *Parus*. The protein data support some, but not all, of the current classification of

parids based on morphology and vocalizations (Snow 1967, Thielcke 1968, Eck 1988). First, the crested North American titmice (subgenus *Baeolophus*) constitute a distinct lineage. The substantial genetic distance (Nei's $\bar{D} = 0.31$) between these titmice and chickadees (subgenus *Poecile*) is comparable to that distinguishing many genera of passerine birds (Johnson et al. 1988). Also, Tufted Titmouse mitochondrial DNA (mtDNA) was markedly different (9%) from the mtDNA of two species of chickadees (Mack et al. 1986). The relationships of these titmice to the superficially similar Gray-crested Tit of the coniferous forests of western China and Tibet warrant study, because this species stands out as perhaps the best candidate for the modern Eurasian representative of this distinct lineage. Thielcke (1968) and Eck (1988) both allied Bridled Tit to the Gray-crested Tit as well as to Crested Tit. Our data establish that Bridled Titmouse is not closely related to Crested Tit ($D = 0.213$), but, instead, may be more closely related to the *Baeolophus* lineage ($D = 0.134, 0.201$). It remains to be resolved whether Bridled Titmouse is more closely related to the *Baeolophus* titmice than to other Eurasian lineages, and how many invasions of North America occurred. The subgenus *Lophophanes* as constituted by Thielcke (1968) is paraphyletic.

The other parid subgenera examined in this study appear to be distinct lineages separated by substantial genetic distances. Coal Tits (subgenus *Periparus*) and Blue Tits (subgenus *Cyanistes*) may be sister lineages, but further study of these two Eurasian species groups is required. We were surprised that Crested Tit appears to be the closest of the Eurasian lineages to *Poecile* chickadees ($\bar{D} = 0.118$, compared to $\bar{D}$s of 0.150 [Coal Tit], 0.220 [Great Tit] and 0.275 [Blue Tit]). To our knowledge, this relationship has not been indicated previously.

Few differences in allozyme compositions were evident among the species of *Poecile* chickadees we examined. Phylogenetic hypotheses among these closely related taxa based on such data are weak and volatile, influenced both by sampling error and specifics of alternative clustering algorithms. A conservative view would be to present the relationships among the North American chickadees as an unresolved polytomy. With this caution, we make the following, potentially controversial observations. Among North American taxa, the close relationships of Mexican Chickadee/Mountain Chickadee and of Chestnut-backed/Boreal were the two clearest results. A derived chromosome arrangement also supports the relationship between Mexican Chickadee and Mountain Chickadee (Holly pers. comm.). The protein data also suggest that: (1) phenotypically confusing (sibling) species, i.e., Carolina/Black-capped chickadees and Marsh/Willow tits, are genetically divergent and are not necessarily sister taxa as we have presumed; (2) the "brown-capped" species (Chestnut-backed, Boreal) are close relatives of the Black-capped Chickadee species group; (3)

the North American taxa probably are more closely related to each other than any is to Willow Tit or Marsh Tit of Eurasia; and (4) the genetic distinction between Black-capped Chickadee and Willow Tit supports earlier conclusions (Snow 1956) that these two taxa are not conspecific and may not be sister taxa.

The lack of genetic differentiation between Pennsylvania and Washington state populations of Black-capped Chickadees is perhaps surprising, given the marked (subspecific) geographical variation in plumage color in this species (Duvall 1945). Our samples from Washington state were of the distinct race *P. a. occidentalis,* not the eastern Washington race *P. a. fortuitus,* which is remarkably similar in appearance to *P. a. atricapillus* from Pennsylvania. Genetic uniformity over such a large region suggests recent geographic expansion of the species (Wake et al. 1978).

One of the principal conclusions evident from these taxonomic comparisons is that species most similar in visual appearance are not necessarily closest genetic relatives. In this regard, perhaps the most controversial result of this protein study pertains to the relationship between the hybridizing species, Black-capped and Carolina chickadees, which are so similar in morphology, vocalizations, and behavior that they are viewed by some as potentially conspecific (Robbins et al. 1986). Distantly related species have converged in the evolution of plumage color patterns and ornamentations, such as crests, which mediate their social interactions. Vocal repertoires may also exhibit such convergence. Head color pattern differences between closely related species, such as Black-capped Chickadee and Mountain Chickadee suggest that such plumage color patterns diverged flexibly and are poor guides to phylogenetic relationships.

Mengel (1964) and Hubbard (1969) developed models of Pleistocene speciation events for North American wood warblers, models which pertain to *Parus* because the evolution of both groups of species is tied to the historical distribution of boreal, cordilleran, and (Pacific) coastal coniferous forests. Mengel's model for wood warblers, however, centered on a Madro-tertiary forest refugium in the southeastern U.S., which was appropriate for some autochthonous New World groups, but may not be fully applicable to an allochthonous Eurasian group such as *Parus.* How many separate invasions are responsible for the modern North American species of chickadees remains unknown. One specific hypothesis (Brewer 1963) is that the ancestor of two species, Black-capped Chickadee and Carolina Chickadee, invaded North America across the Bering land bridge in the late Pliocene or early Pleistocene and separated into an eastern form (Carolina Chickadee) and a western (montane) form (Black-capped Chickadee) during one of the early Pleistocene glaciations. Brewer suggested that expansion of Black-capped Chickadee into the east took place during a subsequent interglacial period, resulting in secondary contact

with Carolina Chickadee, followed by latitudinal shifts in distribution. The protein data suggest a more complex scenario that includes the evolution of Mountain and Mexican chickadees.

Our results are largely consistent with previous allozyme comparisons of parids, e.g., Tufted and Black-crested titmouse (Braun et al. 1984) and Black-capped, Carolina, and Mountain chickadees (Braun and Robbins 1986). The most significant discrepancy was the fixed allelic difference distinguishing Carolina from Black-capped Chickadee at the Gda locus which was not examined by our predecessors. A survey of this locus in both Carolina Chickadee (PA, NJ, GA, N = 33) and Black-capped Chickadee (PA, WA, ONT, N = 26) confirmed this difference in samples from distant localities in the distribution of each species (Gill unpubl. data). The fixed difference at the Gda locus increased the genetic distance between Carolina Chickadee and Black-capped Chickadee from $\bar{D} = 0.001$ (Braun and Robbins 1986) to $\bar{D} = 0.027$. These two species hybridize extensively in a long, narrow zone of contact (Brewer 1963, Rising 1968, Robbins et al. 1986).

We failed to find the differences between Black-capped Chickadee and Mountain Chickadee reported by Braun and Robbins (1986), substantially reducing our estimated genetic distance between these two species. Despite repeated efforts we could not score the locus (Alat-2) at which Braun and Robbins reported a fixed difference. They also found a large frequency difference between Black-capped Chickadee and Mountain Chickadee at Pro-1, but we could not score this locus either. This may have been due merely to differences between laboratories, or to the fact that their sample of Mountain Chickadee was of the distinct California race, *P.g. baileyae,* whereas ours was of the Rocky Mountain race, *P.g. gambeli.* Such discrepancies illustrate how subject to sampling errors allozyme comparisons of closely related taxa may be (Nei 1987).

Two future efforts will provide better resolution of genetic relationships among these chickadees. First will be analyses of mtDNA base pair sequence divergence which enables better discrimination among closely related passerine birds, including species of *Parus,* than do allozymes (Mack et al. 1986, Avise and Zink 1988). The mtDNAs of Black-capped Chickadee and Carolina Chickadee, for example, exhibit a 4% divergence, which suggests separation about two million years ago. Second, genetic comparisons should include Sombre Tit (*P. lugubris*) of Eurasia plus White-browed Tit (*P. superciliosus*) and Pere David's Tit (*P. davidi*) of southwestern China. The White-browed Tit resembles the Mountain Chickadee, and Pere David's Tit may be related to either the Sombre Tit or the Black-capped Chickadee (Eck 1988). Some populations of Sombre Tit (and also of Willow Tit, e.g., the *songarus* group—Vaurie 1959, Snow 1956), have brown caps and resemble Siberian Tits. Any one of these

species could be a close relative of North American taxa. Once these two efforts are complete, and a comprehensive picture of genetic relationships among chickadees is available, we should be able to develop a realistic analogue of Mengel's warbler speciation model for North American parids.

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Appendix I

Alleles Scored at Variable Loci for 15 Species of *Parus* plus *Sitta*. Frequencies of Secondary Alleles are Indicated in Parentheses

E.C. #	Locus	Buffer	Alleles of each species[a]				
			1	2	3	4	5
1.1.1.1	α-glycerophosphate dehydrogenase (αGpdh)	D	e	e	e	e	e
1.1.1.27	Lactate dehydrogenase (Ldh-1,2)	A	a	a	a	a	a
			a	a	a	a	a
1.1.1.37	Malate dehydrogenase (Mdh-2)	B	b	b	b	b	b
1.1.1.40	Malic enzyme (Me)	A	b	b	b	b	b
				c (0.1)	a (0.2)		c (0.3)
1.1.1.42	Isocitrate dehydrogenase (Isdh-1,2)	E	b	b	b	b	b
			c	c	c	c	c
1.1.1.44	Phosphogluconate dehydrogenase (6pgd)	A	b	b	b	b	b
			c (0.1)				
1.15.1.1	Superoxide dimutase (Sod-1,2)	D	a	a	a	a	a
		B	b	b	b	b	b
2.4.2.1	Purine nucleoside phosphorylase (Np)	B	c	c	c	c	c
			b (0.1)	b (0.25)			
2.6.1.1	Aspartate aminotransferase (Aat-1,2)	E	a	a	a	a	a
			b	b	b	b	b
2.7.3.2	Creatine kinase (Ck-2)	B	a	a	a	a	a
2.7.4.3	Adenylate kinase (Adk)	A	c	c	c	c	c
							d (0.1)
2.7.5.1	Phosphoglucomutase (Pgm-1,2,3)	D	b	b	b	b	b
		B	b	b	b	b	b
			c (0.1)				
			c	c	c	c	c
3.1.3.2	Acid phosphatase (Acp)	D	b	b	b	b	b
						a (0.1)	c (0.1)
3.4.11.4	Tripeptide aminopeptidase (Tri-1,2)	A	a	a	a	a	a
			d	d	d	d	d
				b (0.1)			e (0.1)

Appendix I
Continued

Alleles of each species[a]										
6	7	8	9	10	11	12	13	14	15	16
e	e	e	e	e	d	e	d	d	d a (0.2) c (0.2) b (0.1)	c
a	a	a	a	c	a	a	a	a	a	b
a	a	a	a	a	a	a	a	a	a	b
b	b	b	b	b	b	b	b	b	b	a
b	a	b a (0.1)	e	f	e	b	b	a	a	d
b	b	b	b	b a (0.1)	f c (0.1) e (0.1) g (0.1)	b	b	b	b	c d (0.5)
c a (0.4)	c	c	c	c	c	c	c	c	c	b
b	b	b	c	b	c b (0.2)	b	a e (0.2)	a	a	d
a	a	a	a	f	a	a	d	a b (0.5)	e	c
b	b	b	b	b	b	b	b	b	b	a c (0.5)
c	c	c	c	d	d	f g (0.2)	d e (0.1)	d	d	a
a	d	a	a	a	a	a c (0.2)	a	a	a	b
b	b	b	b	b	b	b	b	b	b	a
a	a	a	a	a	a	a	a	a	a	b
c	c	c	c	c	c	c	c	c	c	a b (0.5)
b	b	b	b	b	b	b	b	b	b	a
b	b	b	b	b	b	b	b	b	b	a
c	c	c	c	c	c	c	b a (0.1)	c	c	d
b	b	b	b	b a (0.4)	b	b	b	b	b a (0.1)	b
a	a	a	a	a	a	a	a	a	a c (0.1)	b
d	d	d	d	d b (0.1) f (0.1)	d b (0.4)	d	d	d a (0.1) b (0.2)	d	c

Appendix I
Continued

E.C. #	Locus	Buffer	Alleles of each species[a] 1	2	3	4	5
3.4.13.9	Proline dipeptidase (Pro-2)	B	b a (0.1) c (0.1)	b	b	b	b
3.4.13.11	Dipeptidase (Dip-1,2)	B	c d (0.5) c	c b (0.3) c	c b (0.1) c	c c	d c
3.5.4.3	Guanine deaminase (Gda)	E	a	b	a	a	a
3.5.4.4	Adenosine deaminase (Ada)	A	b	b	b	b	b
5.3.1.8	Mannose phosphate isomerase (Mpi)	A	d b (0.1)	d	d	d	d
5.3.1.9	Glucose phosphate isomerase (Gpi)	C	b	b	b	b	b
———	Myoglobin (Mb)	D	a	a	a	a	a

Monomorphic loci: Sorbitol dehydrogenase (Sordh) [1.1.1.14], Buffer A; Malate dehydrogenase-1 (Mdh-1) [1.1.1.37], Buffer A; Glyceraldehyde-phosphate dehydrogenase (G3pdh) [1.2.1.12], Buffer E; Creatine Kinase-1 (Ck-1) [2.7.3.2), Buffer A; Hemoglobin (Hb), [no E.C. #], Buffer D.

Buffers: A = TC-7.5 (0.2 M tris/0.058 M citric acid/pH 7.5); B = TEB-8.1 (0.2 M tris/0.26 M boric acid/0.005 M EDTA/pH 8.1); C = PC-6 (0.2 M sodium phosphate (monobasic)/0.55 M citric acid/pH 6.0); D = TM-7.5 (0.2 M tris/0.087 M maleic acid/pH 7.5); E = P-7 (0.67 M sodium phosphate (monobasic)/0.133 M sodium phosphate (dibasic).

[a] Species: 1 = *P. atricapillus*; 2 = *P. carolinensis*; 3 = *P. rufescens*; 4 = *P. hudsonicus*; 5 = *P. gambeli*; 6 = *P. sclateri*; 7 *P. palustris*; 8 = *P. montanus*; 9 = *P. ater*; 10 = *P. major*; 11 = *P. caeruleus*; 12 = *P. cristatus*; 13 = *P. wollweberi*; 14 = *P. inornatus*; 15 = *P. bicolor*; 16 = *Sitta carolinensis*.

Appendix I
Continued

Alleles of each species[a]										
6	7	8	9	10	11	12	13	14	15	16
b c (0.4)	b	b	b a (0.2)	b	e f (0.1)	b	b	b c (0.4)	b c (0.2)	d
d	c	c	c	c	c b (0.1)	c	c b (0.2)	c	c b (0.2)	a
c	c	c	a	a	a	a	a	a	a	b
a	a	a	a	a	a	a	a	a	b	c
b	d	d	b	b	b	b	b	c	c	a
d	b	d b (0.1)	e	e	e	g	b f (0.2)	b	b c (0.5)	a
b	b	b	b	b	b	b	a	a	a	a
a	a	a	a	a	a	a	a	a	a	b